CLAIMS

What is claimed is:

1. A method for obtaining improved ligands binding to PIM-1, comprising

determining whether a derivative of a compound that binds to PIM-1 and interacts with one or more of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186 binds to PIM-1 with greater affinity or greater specificity or both than said compound, wherein binding with greater affinity or greater specificity or both indicates that said derivative is an improved ligand.

2. The method of claim 1, wherein said derivative has at least 10-fold greater affinity or specificity or both than said compound.

3. The method of claim 1, wherein said derivative has at least 100-fold greater affinity or specificity or both.

4. The method of claim 1, wherein said compound has a chemical structure of Formula I, Formula II, or Formula III.

5. A method for developing ligands specific for PIM-1, comprising

determining whether a derivative of a compound that binds to a plurality of kinases has greater specificity for PIM-1 than said compound.

6. The method of claim 5, wherein said compound binds to PIM-1 with an affinity at least 10-fold greater than for binding to any of said plurality of kinases.

7. The method of claim 5, wherein said compound interacts with at least one of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186.

8. The method of claim 5, wherein said compound is a compound of Formula I, Formular II, or Formula III.

9. The method of claim 5, wherein said compound binds weakly to said plurality of kinases.

10. A method for developing ligands binding to PIM-1, comprising

identifying as molecular scaffolds one or more compounds that bind to a binding site of PIM-1;

determining the orientation of at least one molecular scaffold in co-crystals with PIM-1; and

identifying chemical structures of said molecular scaffolds, that, when modified, alter the binding affinity or binding specificity or both between the molecular scaffold and PIM-1; and

synthesizing a ligand wherein one or more of the chemical structures of the molecular scaffold is modified to provide a ligand that binds to PIM-1 with altered binding affinity or binding specificity or both.

11. The method of claim 10, wherein said molecular scaffold is a weak binding compound.

12. The method of claim 10, wherein said molecular scaffold binds to a plurality of kinases.

13. The method of claim 10, wherein said molecular scaffold interacts with one or more of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186.

14. The method of claim 10, wherein said molecular scaffold has a chemical structure of Formula 1, Formula II, or Formula III.

15. A method for developing ligands with increased PIM specificity, comprising

testing a derivative of a kinase binding compound for increased PIM specificity, wherein increased specificity is indicative that said derivative is a ligand with increased PIM specificity.

16. The method of claim 15, wherein said kinase binding compound binds to at least 5 different human kinases.

17. The method of claim 15, wherein said kinase binding compound binds to at least 10 different human kinases.

18. The method of claim 15, wherein said PIM is PIM-1, PIM-2, PIM-3, or any combination of at least two of PIM-1, PIM-2, and PIM-3.

19. A method for identifying a ligand binding to PIM-1, comprising

determining whether a derivative compound that includes a core structure selected from the group consisting of Formula I, Formula II, and Formula III binds to PIM-1 with altered binding affinity or specificity or both as compared to the parent compound.

20. A method for determining a structure of a kinase, comprising

creating a homology model from an electronic representation of a PIM-1 structure.

21. The method of claim 20, wherein said creating comprises

identifying conserved amino acid residues between PIM-1 and said kinase;

transferring the atomic coordinates of a plurality of conserved amino acids in said PIM structure to the corresponding amino acids of said kinase to provide a rough structure of said kinase; and

constructing structures representing the remainder of said kinase using electronic representations of the structures of the remaining amino acid residues in said kinase.

22. The method of claim 21, further comprising fitting said homology model to low resolution x-ray diffraction data from one or more crystals of said kinase.

23. The method of claim 21, wherein the coordinates of conserved residues from Table 1 are utilized.

24. The method of claim 21, wherein coordinates of conserved residues from a mutated PIM-1 are utilized.

25. The method of claim 24, wherein said mutated PIM-1 comprises a P123M mutation.

26. A co-crystal of PIM-1 and a PIM-1 binding compound.

27. The co-crystal of claim 26, wherein said binding compound interacts with at least one of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186.

28. The co-crystal of claim 26, wherein said binding compound has structure of Formula I, Formula II, or Formula III.

29. The co-crystal of claim 26, wherein said co-crystal is in an X-ray beam.

30. A crystalline form of PIM-1.

31. The crystalline form of claim 30, having coordinates as described in Table 1.

32. The crystalline form of claim 30, comprising one more more heavy metal atoms.

33. The crystalline form of claim 30, wherein said crystalline form comprises a co-crystal of PIM-1 with a binding compound.

34. The crystalline form of claim 33, wherein said binding compound interacts with one or more of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186.

35. The crystalline form of claim 34, wherein said co-crystal is in an X-ray beam.

36. The crystalline form of claim 30, wherein said crystalline form is in an X-ray beam.

37. The crystalline form of claim 30, wherein said PIM-1 is mutated.

38. The crystalline form of claim 37, wherein said PIM-1 comprises a P123M mutation.

39. A method for obtaining a crystal of PIM-1, comprising subjecting PIM-1 protein at 5-20 mg/ml to crystallization condition substantially equivalent to Hampton Screen 1 conditions 2, 7, 14, 17, 23, 25, 29, 36, 44, or 49 for a time sufficient for cystal development.

40. The method of claim 39, further comprising optimizing said crystallization condition.

41. The method of claim 37, wherein said crystallization condition is selected from the group consisting of 0.2 M LiCl, 0.1 M Tris pH 8.5, 5-15% polyethylene glycol 4000; 0.4-0.9 M sodium acetate trihydrate pH 6.5, 0.1 M imidazole; 0.2-0.7 M. sodium potassium tartrate, 00.1 M MES buffer pH 6.5; and 0.25 M magnesium formate.

42. The method of claim 39, wherein said PIM-1 is seleno-methionine labeled PIM-1.

43. The method of claim 39, wherein said PIM-1 is mutated.

44. The method of claim 43, wherein said PIM-1 comprises a P123M mutation.

45. A method for obtaining co-crystals of PIM-1 with a binding compound, comprising subjecting PIM-1 protein at 5-20 mg/ml to crystallization conditions substantially equivalent to Hampton Screen 1 conditions 2, 7, 14, 17, 23, 25, 29, 36, 44, or 49 in the presence of binding compound for a time sufficient for cystal development.

46. The method of claim 45, wherein said binding compound is added to said protein to a final concentration of 0.5 to 1.0 mM.

47. The method of claim 46, wherein said binding compound is in a dimethyl sulfoxide solution.

48. The method of claim 45, wherein said crystallization condition is 0.4-0.9 M sodium acetate trihydrate pH 6.5, 0.1 M imidazole; or 0.2-0.7 M. sodium potassium tartrate, 00.1 M MES buffer pH 6.5.

49. A method for modulating PIM-1 activity, comprising

contacting PIM-1 with a compound that binds to PIM-1 and interacts with one more of residues 49, 52, 65, 67, 121, 128, and 186.

50. The method of claim 49, wherein said compound is a compound of Formula I, Formula II, or Formula III.

51. The method of claim 49, wherein said compound is at a concentration of 200 μM or less.

52. A method for treating a patient suffering from a disease or condition characterized by abnormal PIM-1 activity, comprising

administering to said patient a compound that interacts with one or more of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186.

53. The method of claim 52, wherein said compound is a compound of Formula I, Formula II, or Formula III.

54. The method of claim 50 wherein said disease or condition is a cancer.

55. The method of claim 52, wherein said disease or condition is an inflammatory disease or condition.

56. An electronic representation of a crystal structure of PIM-1.

57. The electronic representation of claim 56, containing atomic coordinate representations corresponding to the coordinates listed in Table 1.

58. The electronic representation of claim 56, comprising a schematic representation.

59. The electronic representation of claim 56, wherein atomic coordinates for a mutated PIM-1 are utilized.

60. The electronic representation of claim 59, wherein said mutated PIM-1 comprises a P123M mutation.

61. The electronic representation of claim 59, containing atomic coordinate representations corresponding to the coordinates listed in Table 1 modified by the replacement of coordinates for proline at position 123 by coordinates for methionine.

62. An electronic representation of a binding site of PIM-1.

63. The electronic representation of claim 62, comprising representations of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186.

64. The electronic representation of claim 62, comprising a binding site surface contour.

65. The electronic representation of claim 62, comprising representations of the binding character of a plurality of conserved amino acid residues.

66. The electronic representation of claim 62, further comprising an electronic representation of a binding compound in a binding site of PIM-1.

67. The electronic representation of claim 62, wherein said PIM-1 is a mutated PIM-1.

68. The electronic representation of claim 67, wherein said PIM-1 is mutated by the replacement of proline at position 123 by methionine.

69. An electronic representation of a PIM-1 based homology model for a kinase.

70. The electronic representation of claim 69, wherein said homology model utilizes conserved residue atomic coordinates of Table 1.

71. The electronic representation of claim 69, wherein atomic coordinates for a mutated PIM-1 are utilized.

72. The electronic representation of claim 71, wherein said mutated PIM-1 comprises a P123M mutation.

73. An electronic representation of a modified PIM-1 crystal structure, comprising an electronic representation of the atomic coordinates of a modified PIM-1.

74. The electronic representation of claim 73, comprising the atomic coordinates of Table 1, modified by the replacement of atomic coordinates for proline with atomic coordinates for methionine at PIM-1 residue 123.

75. The electronic representation of claim 73, wherein said modified PIM-1 comprises a C-terminal deletion, an N-terminal deletion or both.

76. A method for developing a biological agent, comprising

analyzing a PIM-1 structure and identifying at least one sub-structure for forming a said biological agent.

77. The method of claim 76, wherein said substructure comprises an epitope, and said method further comprises developing antibodies against said epitope.

78. The method of claim 76, wherein said sub-structure comprises a mutation site expected to provide altered activity, and said method further comprises creating a mutation at said site thereby providing a modified PIM-1.

79. The method of claim 76, wherein said sub-structure comprises an attachment point for attaching a separate moiety.

80. The method of claim 79, wherein said separate moiety is selected from the group consisting of a peptide, a polypeptide, a solid phase material, a linker, and a label.

81. The method of claim 79, further comprising attaching said separate moiety.

82. A method for identifying potential PIM-1 binding compounds, comprising

fitting at least one electronic representations of a compound in an electronic representation of a PIM-1 binding site.

83. The method of claim 82, wherein said electronic representation of a PIM-1 binding site is defined by atomic structural coordinates set forth in Table 1.

84. The method of claim 83, comprising

removing a computer representation of a compound complexed with PIM-1 and fitting a computer representation of a compound from a computer database with a computer representation of the active site of PIM-1; and

identifying compounds that best fit said active site based on favorable geometric fit and energetically favorable complementary interactions as potential binding compounds.

85. The method of claim 83, comprising

modifying a computer representation of a compound complexed with PIM-1 by the deletion or addition or both of one or more chemical groups;

fitting a computer representation of a compound from a computer database with a computer representation of the active site of PIM-1; and

identifying compounds that best fit said active site based on favorable geometric fit and energetically favorable complementary interactions as potential binding compounds.

86. The method of claim 83, comprising

removing a computer representation of a compound complexed with PIM-1 and; and

searching a database for compounds having structural similarity to said compound using a compound searching computer program or replacing portions of said compound with similar chemical structures using a compound construction computer program.

87. The method of claim 83, wherein said compound complexed with PIM-1 is a compound of Formula I, Formula II, or Formula III.

88. The method of claim 82, wherein said fitting comprises determining whether a said compounds will interact with one or more of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186.

89. A method for attaching a kinase binding compound to an attachment component, comprising

identifying energetically allowed sites for attachment of a said attachment component on a kinase binding compound; and

attaching said compound or derivative thereof to said attachment component at said energetically allowed site.

90. The method of claim 89, wherein said attachment component is a linker for attachement to a solid phase medium, and said method further comprises attaching said compound or derivative to a solid phase medium through a linker attached at a said energetically allowed site.

91. The method of claim 89, wherein said kinase is PIM-1 kinase.

92. The method of claim 89, wherein said kinase comprises conserved residues matching at least one of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186.

93. The method of claim 90, wherein said linker is a traceless linker.

94. The method of claim 90, wherein said kinase binding compound or derivative thereof is synthesized on a said linker attached to said solid phase medium.

95. The method of claim 94, wherein a plurality of said compounds or derivatives are synthesized in combinatorial synthesis.

96. The method of claim 90, wherein attachment of said compound to said solid phase medium provides an affinity medium.

97. The method of claim 89, wherein said attachment component comprises a label.

98. The method of claim 97, wherein said label comprises a fluorophore.

99. A modified compound, comprising

a compound of Formula I, Formula II, or Formula III, with a linker moiety attached thereto.

100. The compound of claim 99, wherein said linker is attached to an energetically allowed site for binding of said modified compound to PIM-1.

101. The compound of claim 99, whereins said linker is attached to a solid phase.

102. The compound of claim 99, wherein said linker comprises or is attached to a label.

103. The compound of claim 99, wherein said linker is a traceless linker.

104. A modified PIM-1 polypeptide, comprising a P123M modification.

105. The modified PIM-1 polypeptide of claim 104, wherein said polypeptide comprises a full-length PIM-1 polypeptide.

106. The modified PIM-1 polypeptide of claim 104, wherein said polypeptide comprises a modified PIM-1 binding site.

107. The modified PIM-1 polypeptide of claim 104, wherein said polypeptide comprises at least 50 contiguous amino acid residues derived from PIM-1 sequence including said P123M modification.

108. The modified PIM-1 polypeptide of claim 104, comprising a full-length PIM-1.

109. A method for developing a ligand for a kinase comprising conserved residues matching one or more of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186, comprising

determining whether a compound of Formula I, Formula II, or Formula III binds to said kinase.

110. The method of claim 109, wherein said kinase comprises conserved residues matching at least 2 of PIM-1 residues 49, 52, 65, 67, 121, 128, and 186.

111. The method of claim 109, wherein said kinase comprises conserved residues matching PIM-1 residues 49, 52, 65, 67, 121, 128, and 186.

112. The method of claim 109, further comprising determining whether said compound modulates said kinase.

113. The method of claim 109, wherein said determining comprises computer fitting said compound in a binding site of said kinase.

114. The method of claim 109, further comprising forming a co-crystal of said kinase and said compound.

115. The method of claim 114, further comprising determining the binding orientation of said compound with said kinase.

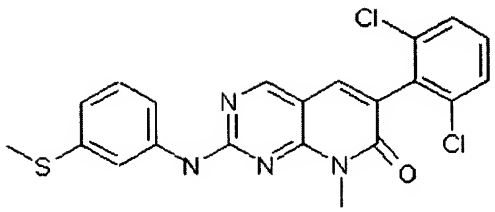
116. The method of claim 109, wherein said kinase has at least 25% sequence identity to full-length PIM-1.

117. A method for treating a PIM-1 associated disease, comprising

administering to a patient suffering from or at risk of a PIM-1 associated disease a therapeutic amount of a 2-phenylaminopyrimidine compound or a pyrido-[2,3-d]pyrimidine compound.

118. The method of claim 117, wherein said compound is imatinib mesylate or derivative thereof.

119. The method of claim 117, wherein said compound is



or a derivative thereof.